

Landfill sludge treatment: a proposal based on the circular bioeconomy perspective

Tratamento de lodo de aterro: uma proposta baseada na perspectiva da bioeconomia circular

Tratamiento de lodos de basurero: una propuesta desde la perspectiva de la bioeconomía circular

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Abstract

The cultivation of microalgae in liquid residues appears as a biotechnological possibility due to the sustainability of the process, since pollutants present in residues such as nitrogen (N) and phosphorus (P) are used by these microorganisms as nutrients for their growth and biomass production with added value. The objective of this work was to develop a prototype of photobioreactors to remove nutrients: nitrogen and phosphorus through the cultivation of microalgae *Chlorella vulgaris* in landfill leachate diluted in domestic effluent. The cultivation lasted 28 days where the removal of nutrients nitrogen and phosphorus was evaluated, as well as microalgal growth. The best results of algal growth were obtained in photobioreactors containing 100% domestic effluent and 15% slurry with chlorophyll concentration of $2.277,08 \pm 247,21 \mu\text{g.L}^{-1}$ and $223,12 \pm 17,44 \mu\text{g L}^{-1}$, respectively, on the 21st day of culture. Regarding the removal of nutrients of nitrogen and phosphorus, it was possible to observe that the species used in this study showed good efficiency, about 72,36% for nitrogen and 44,32% for phosphorus at the end of the cultivation time.

Keywords: Biotechnology; Circular economy; Leachate landfill; Microalgae; Wastewater.

Resumo

O cultivo de microalgas em resíduos líquidos surge como uma possibilidade biotecnológica devido à sustentabilidade do processo, uma vez que poluentes presentes em resíduos como nitrogênio (N) e fósforo (P) são utilizados por esses microrganismos como nutrientes para seu crescimento e produção de biomassa com valor adicionado. O objetivo deste trabalho foi desenvolver um protótipo de fotobiorreatores para remoção de nutrientes: nitrogênio e fósforo através do cultivo da microalga *Chlorella vulgaris* em lixiviado de aterro sanitário diluído em efluente doméstico. O cultivo teve duração de 28 dias onde foi avaliada a remoção de nutrientes nitrogênio e fósforo, bem como o crescimento de microalgas. Os melhores resultados de crescimento de microalgas foram obtidos em fotobiorreatores contendo 100% de efluente doméstico e 15% de chorume com concentração de clorofila de $2.277,08 \pm 247,21 \mu\text{g.L}^{-1}$ e $223,12 \pm 17,44 \mu\text{g.L}^{-1}$, respectivamente, no 21^o dia de cultivo. Com relação à remoção de nutrientes de nitrogênio e fósforo, foi possível observar que a espécie utilizada neste estudo apresenta boa eficiência, cerca de 72,36% para nitrogênio e 44,32% para fósforo ao final do cultivo.

Palavras-chave: Biotecnologia; Economia circular; Aterro sanitário; Microalgas; Águas residuais.

Resumen

El cultivo de microalgas en residuos líquidos aparece como una posibilidad biotecnológica debido a la sostenibilidad del proceso, ya que los contaminantes presentes en los residuos como el nitrógeno (N) y el fósforo (P) son utilizados por estos microorganismos como nutrientes para su crecimiento y producción de biomasa con valor añadido. El objetivo de este trabajo fue desarrollar un prototipo de fotobiorreactores para la remoción de nutrientes: nitrógeno y

fósforo mediante el cultivo de la microalga *Chlorella vulgaris* en lixiviados de vertedero diluidos en efluentes domésticos. El cultivo tuvo una duración de 28 días donde se evaluó la remoción de nutrientes nitrógeno y fósforo, así como el crecimiento de microalgas. Los mejores resultados de crecimiento de algas se obtuvieron en fotobiorreactores que contenían 100% efluente doméstico y 15% purín con concentración de clorofila de $2.277,08 \pm 247,21 \mu\text{g.L}^{-1}$ y $223,12 \pm 17,44 \mu\text{g.L}^{-1}$, respectivamente, en el 21 día de la cultura. En cuanto a la remoción de nutrientes de nitrógeno y fósforo, se pudo observar que las especies utilizadas en este estudio mostraron una buena eficiencia, alrededor de 72,36% para nitrógeno y 44,32% para fósforo al final del tiempo de cultivo.

Palabras clave: Biotecnología, Economía circular, Vertedero de lixiviados; Microalgas; Aguas residuales.

1. Introduction

The increase in consumption has a direct impact the increase in the generation of municipal solid waste, which mostly does not have adequate disposal after its disposal, which represents environmental impacts and a challenge for public administration to develop adequate management and final disposal of waste (Luo, et al., 2020). The gravimetric composition of solid waste generated in Brazil is equivalent to 51.4% of organic matter, about 2,050 tons of solid waste are produced daily in the city of Salvador, Ba (IBGE-ALBREPE, 2010).

Article 9 of Lei No. 12,305/2010 of the National Politics on the action of Solid Waste, the waste deposit in the soil is the last way to be used, however in Brazil, much of this waste is transported to dumps, controlled landfills and landfills more commonly than due (ABRELPE, 2015). Although some landfills are designed so that the environmental impacts generated are minimal, a slurry is a major challenge, because due to its composition and low degradability, it can negatively impact public health, soil, rivers and groundwater (Luo, et al., 2020; Luz, et al., 2020; MMA, 2005).

The Slurry is a liquid effluent, generated in the decomposition of the organic fraction and municipal solid waste, also known as landfill leaching, have high turbidity, acid pH, high Biochemical Oxygen Demand (BOD), high value of Chemical Oxygen Demand (COD), a high concentration of nitrogen and heavy metals (Yan, et al., 2015; Costa, et al., 2019). The technologies developed for the removal of slurry contaminants have a high cost as well as are relatively limited due to their composition, are based on biological, physical and chemical processes and the combination of biological and physicochemical for better efficiency in treatment, however, little attention is given to recycling nutrients (Miao, et al., 2019), on biological, chemical and physical process (Costa, et al., 2019; Chen, et al., 2018).

In this context, the circular bioeconomy arises to explore waste as inputs in biotechnological processes. Aiming at the best use of waste, a biotechnological system based on microalgae is considered promising for the treatment of landfill leaching (slurry), since these microorganisms use nitrogen and phosphorus presents in leached as a source of nutrients for their growth and production of biomass (Quan, et al., 2020).

Microalgae are aquatic microorganisms, photosynthesizer, which transform light, carbon dioxide present in the atmosphere and the nutrients present in the water into biomass rich in different molecules, such as proteins, carbohydrates, lipids, vitamins and antioxidants (Marques, et al., 2021). These biomass components are used for the production of bioproducts such as: biodiesel (Dogaris, et al., 2020), bioethanol (Maia, et al., 2020), bioplastics (Chong, et al., 2022) biomethane (Xiao, et al., 2019), biohydrogen (Batista, et al., 2015), bioelectricity (Maity, et al., 2014).

Therefore, the objective of this work was to investigate the viability of cultivation and the adaptation of *Chlorella vulgaris* microalgae in landfill slurry diluted in domestic effluent, in the prototype photobioreactor, as well as its efficiency in the removal of pollutants: nitrogen and phosphorus.

2. Materials and Methods

This research was carried out in the Laboratory of University Salvador, Brazil and partnership with the Petroleum Studies Laboratory (LEPETRO) of the Federal University of Bahia, Brazil (IGEO/UFBA).

2.1 Obtaining microalgal strain

The microalgae strain of the species *Chlorella vulgaris* was acquired from the Canadian Phycological Culture Centre (CPCC). The culture medium used for serial propagation of this species was autoclaved at 121°C for 15 minutes for sterilization. The microalgae were initially inoculated in Wright's Cryptophyte Medium – WC (Andersen et al., 2005) to stimulate its growth according to the methodology proposed by reference (Andrade & Filho, 2014), whose nutrient composition is by Table 1.

Table 1 - Composition of the Wright's Cryptophyte Medium - WC used for the cultivation of microalgae

Reagents	Solution -stock	Culture medium (mL)
In ₂ SiO ₃ •5H ₂ O	21,2 g/100 ml	1 mL
CaCl ₂ •2H ₂ O	36,8 g/100 ml	1 mL
MgSO ₄ •7H ₂ O	37.0 g/100 ml	1 mL
NaNO ₃	85 g/100ml	1 mL
K ₂ HPO ₄	8,7 g/100 ml	1 mL
NaHCO ₃	12.6 g/100 ml	1 mL
Iron Solution	(g.L ⁻¹ distilled water); Na ₂ EDTA = 4.36; FeCl ₃ .H ₂ O = 3.15	1 mL
Micronutrient Solution	(g.L ⁻¹ distilled water) CuSO ₄ .5H ₂ O = 0.01; ZnSO ₄ .7H ₂ O = 0.022; CoCl ₂ .H ₂ O = 0.01; MnCl ₂ .4H ₂ O= 0.18; Na ₂ MoO ₄ .2H ₂ O = 0.006; H ₃ BO ₃ = 1.0	1 mL
Vitamin solution	(g.L ⁻¹ distilled water) Thiamine HCl= 0.1; Biotin= 0.0005	1 mL
Distilled water	-----	1mL

Source: Andersen et al. (2005); Andrade e Filho (2014).

2.2 Acquisition of slurry samples

The landfill leached (Slurry) used in this study collected in the installations of Bahia Transferência e Tratamento de Resíduos (BATTRE) landfill in Salvador City, Bahia, Brazil. The wastewater commonly known as domestic effluent was collected upstream of the effluent treatment and wastewater treatment plant - ETE Muriçoca of the Empresa Bahia de Águas e Saneamento S.A – EMBASA in the Salvador City, Bahia, Brazil. 8 L of slurry and 24 L of domestic effluent were collected, the samples were packed in amber bottles with a volume of 4L, stored in thermal boxes and sent to the laboratory.

2.3 Cultivation Conditions

Initially, the leach samples were homogenized in the proportions of 15%, 30% and 50% with domestic effluent, then autoclaved at 121 °C for 15 minutes for sterilization. The cultivation was carried out in triplicate with a temperature of 23°C, using 3 L photobioreactors with a useful volume of 2.5 L under aeration with the aid of 3W aquarium pumps and 3L min⁻¹ flow to keep microalgae in suspension and provide CO₂.

The experiment was carried out in a closed system, and the air was filtered through a 0.22 µm filter. The illuminance was performed with cold fluorescent lamps with a power of 11W with approximately 1400 lux and absence of photoperiod. The photobioreactors were named as WC culture medium, 15% slurry, 30% slurry, 50% slurry and 100% domestic effluent. Figure 1 shows the experimental design of the prototype and the respective concentrations of the photobioreactors according to the proportions described in Table 2:

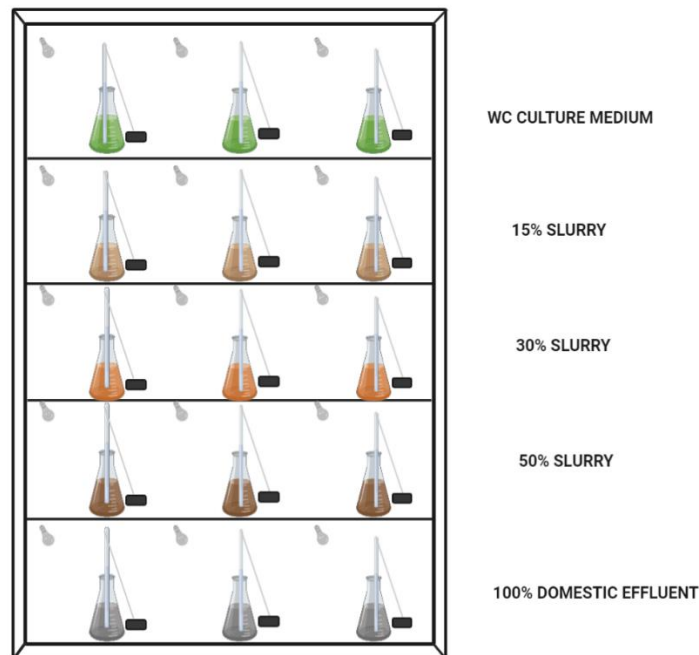
Table 2 - Proportion of concentrations of photobioreactors.

Concentration	Slurry	Domestic Effluent
WC culture medium	0%	0%
15% slurry	15%	85%
30% slurry	30%	70%
50% slurry	50%	50%
100% domestic effluent	0%	100%

Source: Authors (2022).

The experiment was monitored for 28 days, is divided into 6 different collected times: 0d, 1d, 7d, 14d, 21d and 28d to temporally analyze nutrient removal and microalgal growth. 0d was considering only the assembly of the experiment without adding the microalgae strains. The 1d was considered as the transfer of the microalgae strains to the experiment being the first day of cultivation. Temperature, luminosity and pH were monitored daily by a digital thermometer (TPM-10), digital lux meter (AKSO) and the brand's pH measuring strips (MColorpHast™) when necessary, the pH correction of the crop was made.

Figure 1 - Experimental design of the photobioreactor system according to the five gradients of different concentrations.



Source: Authors (2022).

2.4 Laboratory Analyses

In the laboratory, the samples collected temporally were filtered using a microfiber glass filter membrane (GF/A 47mm) containing pore size of 0.45 μ m, with the aid of a vacuum pump.

2.5 Determination of total nitrogen residual concentration Kjeldahl

The determination of the residual concentration of total nitrogen according to the method proposed by reference (APHA, 1998) With the aid of a volumetric pipette, 50.0 mL of the sample was collected and transferred to a Kjeldahl balloon.

After the transfer, the pH of the sample was neutralized for 7. To maintain the pH at 7.4 during distillation, 6.5 mL of phosphate buffer solution was added. The sample was diluted to about 100.0 mL with distilled water and about 50 mL of the diluted solution was styled. The Erlenmeyer used for the collection of the diluted sample contained 50 mL of boric acid indicator solution, in which the output end of the distillate was immersed in this indicator solution. White distillation was performed containing all reagents used, replacing 50.0 mL of sample with 50.0 mL of distilled water. The Erlenmeyer content was titrated with sulfuric acid 0.02 N as titrant until the turn of the indicator (green for violaceous). Titration was repeated for the white test distillate. The determination of total nitrogen residual concentration was calculated according to Equation 1.

$$N - NKT = \frac{(VA_{H_2SO_4} - VB_{H_2SO_4})N_{H_2SO_4} \times 14000}{\text{sample volume}} \left(\frac{\text{mg}}{\text{L}} \right) \quad (1)$$

VA_{H₂SO₄} = volume of sulfuric acid spent on titration (mL)

VB_{H₂SO₄} = volume of sulfuric acid spent on white (mL)

N_{H₂SO₄} = normal sulfuric acid

14000 = nitrogen conversion factor of the unit of liter equivalent number to mg L⁻¹

Sample volume = sample rate taken for digestion (mL)

2.6 Determination of residual concentration of assimilated Phosphorus

The determination of the assimilated residual Phosphorus (P) concentration according to the method proposed by reference (APHA, 1998). Initially, 2 mL of the filtered sample (5 mL filtered as described in item 2.5) was transferred to the falcon tubes, subsequent transfer to dilution of the sample, 10 mL of Milli-Q water after the dilution added 0,2 mL of the ascorbic acid solution 10 g.L⁻¹ to the sample. 0,2 mL of the combined reagent (molybdate + tartrate) was added. After 10 minutes, waiting time for the formation of the blue complex, the sample was transferred to a 1cm bucket and subsequent reading in a spectrophotometer (CARY 60 UV-Vis) in absorbance of 880 nm. The residual concentration of assimilable phosphorus was calculated according to Equation 2.

$$PO_4^{-3} = (CP_{PO_4^{-3}} - CB) \times \text{Number of dilution times} \left(\frac{\text{mg}}{\text{L}} \right) \quad (2)$$

CP: partial concentration of PO₄⁻³ (mg L⁻¹)

CB: white concentration (mg L⁻¹)

2.7 Nitrogen and phosphorus removal efficiency

The efficiency of nitrogen (N) and phosphorus (P) removal was analyzed by calculations according to Equations 3 and 4 respectively according to the reference (Nayak, et al., 2016).

$$\mu N(\%) = \frac{Ni - Nf}{Ni} \times 100 \quad (3)$$

$$\mu P(\%) = \frac{Pi - Pf}{Pi} \times 100 \quad (4)$$

Nf: final reading of the total amount of nitrogen in the sample

Ni: initial reading of the total amount of nitrogen in the sample

Pi: initial reading of the total amount of phosphorus in the sample

Pf: final reading of the total amount of phosphorus in the sample

2.8 Microalgae Growth

The cell concentration was evaluated by the method of analysis of chlorophyll (*a*) which is a pigment extracted from microalgae and submitted to reading and spectrophotometer (CARY 60 UV-Vis) at wavelengths at 630 nm, 647 nm, 664 nm and 750 nm. The quantification of microalgae was performed through calculations described by the reference (APHA, 2012) referring to the concentration of chlorophyll(*a*), where the optical density values established by the extraction were inserted, according to Equation 3. After the extraction concentration was determined, the total number of cells per volume unit was calculated, according to Equation 5, the result was converted from mg/m³ to µg L⁻¹.

$$Ca = 11,85 (\text{absorbancia}_{664}) - 1,54((\text{absorbancia}_{647}) - 0,08(\text{absorbancia}_{630})) \quad (5)$$

$$\text{Chlorophyll a} = \frac{Ca \cdot \text{volume extraction (L)}}{\text{Sample volume (m}^3\text{)}} \left(\frac{\text{mg}}{\text{m}^3} \right) \quad (6)$$

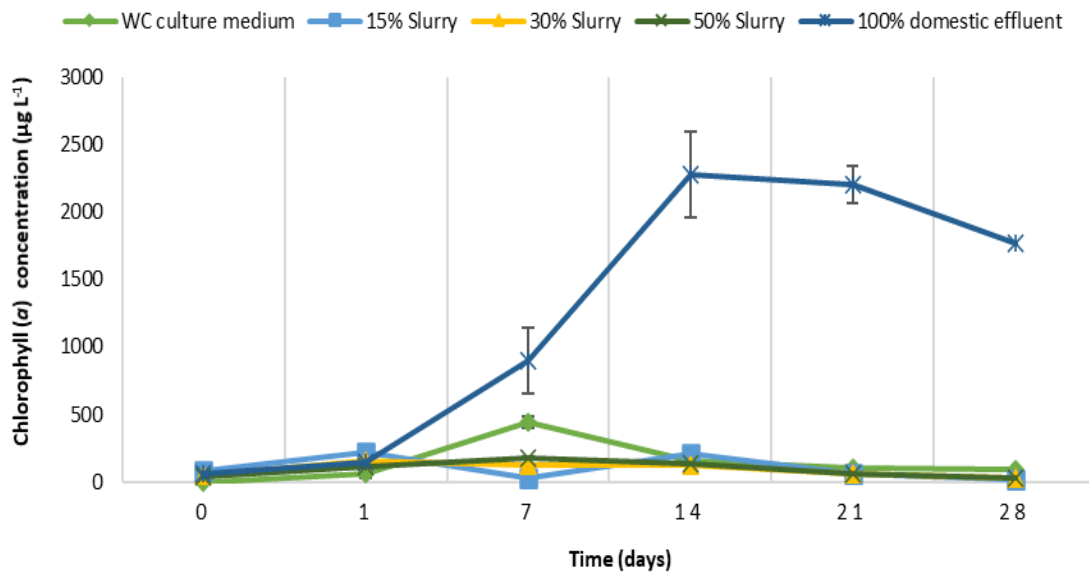
3. Results and Discussion

3.1 Microalgae Growth

The growth of *Chlorella vulgaris* microalgae grown in WC culture medium and different concentrations of diluted slurry in domestic effluent over the 28 days of cultivation, in the 6 different collection times is represented in Figure 2 (A) where it represents the growth of the microalgae in different concentrations of slurry and in 100% of domestic effluent for comparison. The Figure 2(B) represents only the growth of the microalgae in different concentrations of slurry. The mean concentrations of chlorophyll (*a*) and standard deviation, are described according to Table 3. Time 0 day corresponds to the initial chlorophyll (*a*) concentration according to each dilution without the addition of the microalgal strain. The strain of microalgae was transferred to the photobioreactors on the 1st day of cultivation, this time corresponds to the phase of adaptation of the microorganism to the new inserted medium, since they were inoculated in an ideal culture medium for its growth.

Exponential growth corresponds to the phase of higher metabolic activity, occurs after the adaptation phase. It is possible to observe this phase started after the 1st day until the 21st day, this exponential growth is linked to the concentration of nutrients available in the medium. To algal growth, it can be observed in Figure 2(A) and Table 3 that regardless of the concentrations there was the growth of microalgae, the culture cultivated in 100% domestic effluent showed the highest concentration of chlorophyll (*a*) with about 2.277,08±247,21 µg.L⁻¹ on the 21st day. This fact may be linked to a better condition of light penetration in the crop and consequently of photosynthesis, besides presenting bioavailability of nutrients (Iasimone, et al., 2018).

Figure 2(A) - Monitoring *Chlorella vulgaris* microalgae growth in different slurry concentrations and the photobioreactor containing 100% domestic effluent.

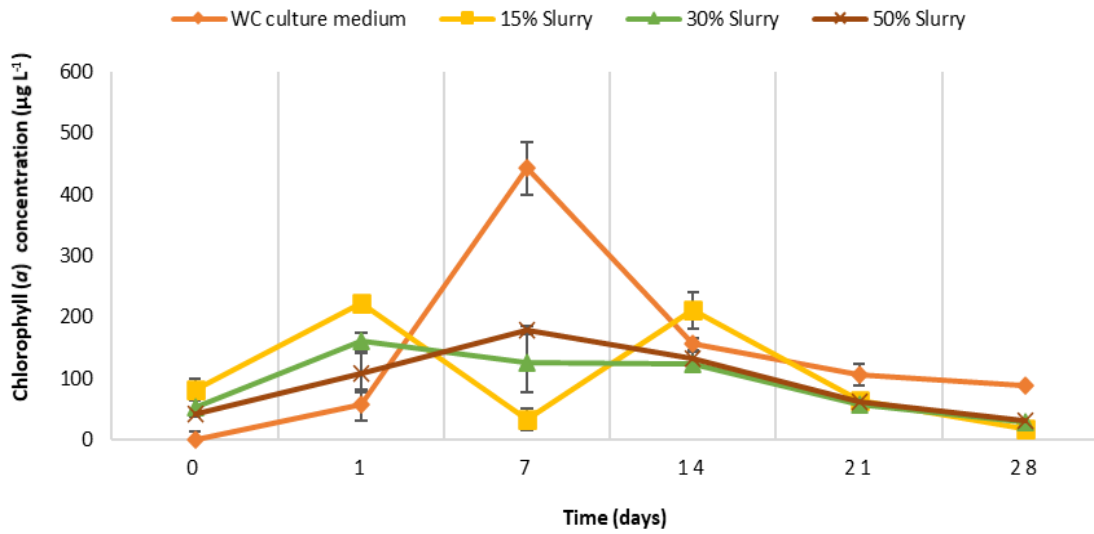


Source: Authors (2022).

Against the basis of the crops grown in a medium containing the slurry concentrations (Figure 2(B) and Table 3), the best growth was presented in the concentration of 15% slurry with $223,12 \pm 17,44 \mu\text{g.L}^{-1}$ on the 1st day, which suggests that there was the influence of light penetration in the growth conditions and a balance of the distribution of nutrients of interest of microalgae. The growth behavior of microalgae in the cultures of this study differs from that presented in the literature, microalgae did not show growth in crops with slurry concentrations above 10% (Barbosa, et al., 2017), however, in the study by Barbosa et al. (2017) the microalgae were cultivated in concentrations of slurry diluted in distilled water. In the present study, dilution of slurry with domestic effluent was established precisely to provide a balance in nutrient bioavailability, since the slurry has a higher concentration of ammoniacal nitrogen and almost null for phosphorus. Another study that used *Scenedesmus sp.* microalgae in different dilutions of slurry with domestic effluent, highlighted that its growth increased from 5% to 15% dilution, also caused by lower light penetration (Saleem, et al., 2022).

Conform shows Figure 2(B) and Table 3, in this study, microalgae growth in leached was obtained in concentrations containing up to 50% slurry. This fact can be justified by the increment of a phosphate source, arising from the domestic effluent that corroborated for a satisfactory result of microalgal growth, presenting as a result maximum growths of: $211,47 \pm 18,56$ in 15% Slurry, $126,30 \pm 14,89$ at 30% Slurry and $178,00 \pm 31,97$ at 50% Slurry.

Figure 2(B) - Monitoring *Chlorella vulgaris* microalgae growth only in different slurry concentrations.



Source: Authors (2022).

The phase growth decline was observed after the 7st day, which is directly related to the decrease in the availability of some essential nutrients for the growth of microorganisms because as there is the growth of microalgae density over time, there is nutrients consumption and also the reduction of light penetration resulting from self-shading causing stress (Lam, et al., 2017). Previous studies Hu et al. (2021) demonstrate that the leachate dilution rate of 10% showed a higher concentration of *Chlorella vulgaris* microalgae biomass, considering that the leachate was diluted and pre-treated. Therefore, the domestic effluent not only provided nutrients such as phosphorus to the culture medium, but also reduced the turbidity of the slurry, allowing light penetration and microalgae growth.

Table 3 - Average chlorophyll (α) concentrations ($\mu\text{g.L}^{-1}$). Data with mean and standard deviation (n=3).

Concentrations	0 day	1 st day	7 th day	14 th day	21 st day	28 th day
WC culture medium	0,05 $\pm 0,01$	56,63 $\pm 14,03$	442,44 $\pm 25,53$	156,08 $\pm 42,73$	105,44 $\pm 9,70$	87,94 $\pm 18,05$
15% Slurry	81,85 $\pm 0,64$	223,12 $\pm 17,44$	32,81 $\pm 2,39$	211,47 $\pm 18,56$	62,69 $\pm 29,75$	16,46 $\pm 0,99$
30% Slurry	52,76 $\pm 3,53$	160,03 $\pm 16,19$	126,30 $\pm 14,89$	123,38 $\pm 48,67$	56,20 $\pm 2,38$	28,72 $\pm 2,07$
50% Slurry	41,13 $\pm 7,52$	108,82 $\pm 2,33$	178,00 $\pm 31,97$	132,93 $\pm 6,94$	62,48 $\pm 9,65$	30,19 $\pm 6,50$
100% domestic effluent	63,13 $\pm 2,27$	143,44 $\pm 11,35$	897,92 $\pm 63,02$	2277,08 $\pm 247,21$	2209,26 $\pm 318,86$	1767,33 $\pm 137,35$

Source: Authors (2022).

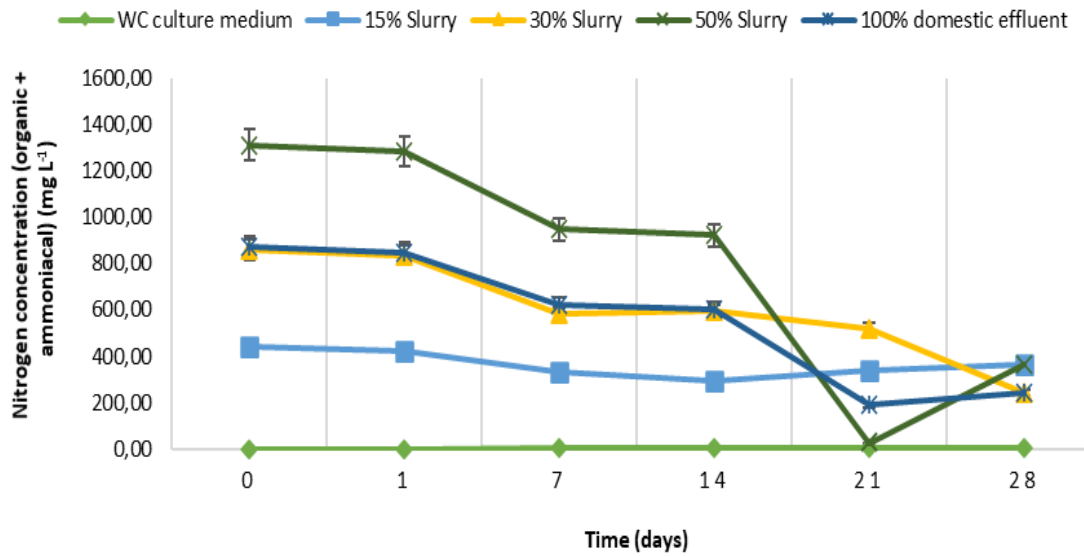
3.2 Nitrogen Removal

Nitrogen is a necessary macronutrient for the metabolism of microorganisms, whether microalgae or cyanobacteria, where it contributes to the formation of proteins, however, the reduction of nitrogen concentration allows lipids and carbohydrates to be synthesized primarily (Duarte, et al., 2021). In general, microalgae can assimilate nitrogen from such as: ammonia, nitrate, nitrite and urea (Wang, et al., 2016). The highest nitrogen concentration was initially presented in 50% slurry containing $1.288,00 \pm 2,67 \text{ mg.L}^{-1}$ reducing to $364,00 \pm 2,00$ after 21 days. For photobioreactors containing 15% and 30% slurry, they present results of initial nitrogen concentrations with $423,00 \pm 1,20 \text{ mg.L}^{-1}$ and $838,00 \pm 6,67 \text{ mg.L}^{-1}$ reducing to $363,00 \pm 4,22 \text{ mg.L}^{-1}$ and $241,67 \pm 3,78 \text{ mg.L}^{-1}$, respectively after 28 day cultivation. Regarding 100% domestic effluent, a concentration of $849,67 \pm 3,58 \text{ mg.L}^{-1}$ of nitrogen was presented, reducing to $241,67 \pm 3,78 \text{ mg.L}^{-1}$.

Analyzing a Figure 3, it is noted that there was a gradual decrease in the concentration of nitrogen available in the medium, occurred in the first 7 days, demonstrating the consumption of the nutrient by the microalgae and the increased the Slurry curve 50% after the 21st day, this can be explained by the transformation of another nitrogen source, that is, the decomposition of organic nitrogen into ammonia through ammonia, which was then can be transformed into nitrate and nitrite through nitrification (Chen, et al., 2018).

According to the reference (Ruiz-Marin, et al., 2010), *Chlorella vulgaris* microalgae shows a preference for ammonia over any other form of nitrogen. Ammonia is the most energy efficient nitrogen source because it has the lowest molecular weight compared to other forms of nitrogen, less energy is needed for its absorption through microalgae.

Figure 3 - Nitrogen concentration (Organic and ammoniacal) over the 28 days of microalgae cultivation according to the different concentrations of slurry and domestic effluent.



Source: Authors (2022).

3.3 Discussion of nitrogen removal and efficiency

The main sources of nitrogen in landfill leaching are of plant or animal origin, their concentration is directly related to the amount of organic matter present in the residues. Other nitrogen sources that may be present in leached are: fertilizers, cleaning products, meat preserved with ammonia and wood preservation products (Li, et al., 2022). The physicochemical analyses made by BATTRE prove the existence of a high concentration of ammoniacal nitrogen in the slurry, about 2.032 mg.L⁻¹. It is possible to observe that even being different contractions the efficiency in nitrogen removal was almost uniform about 72,29%, 72.36% and 71.93% of removal occurring in crops: 50% slurry, 100% domestic effluent and 30% slurry respectively.

Studies such as Li et al. (2022) using *Chlorella vulgaris* evaluated the nitrogen removal rate in slurry, found a removal of 65% due to the influence of the N:P ratio, which was 190:1. Studies indicate that a balanced N/P ratio is required for slurry removal, considering 90:1 to 30:1 (Paskuliakova et al. 2016). In the present study, the N/P ratio was found to be 19.27:1.

Table 4 - Nitrogen removal (mg L⁻¹) and efficiency considerer different slurry concentrations after 28 days of microalgae cultivation. Data with mean and standard deviation (n=3).

Concentrations	Initial Nitrogen (mg.L ⁻¹)	Final Nitrogen (mg.L ⁻¹)	Efficiency (%)
WC culture medium (Control)	7,87±0,01	7,72±0,32	1,91%
15% Slurry	423,00±1,20	363,00±4,22	18,00%
30% Slurry	838,00±6,67	241,67±3,78	71,93%
50% Slurry	1288,00±2,67	364,00±2,00	72,36%
100% domestic effluent	849,67±3,58	241,67±3,78	72,29%

Source: Authors (2022).

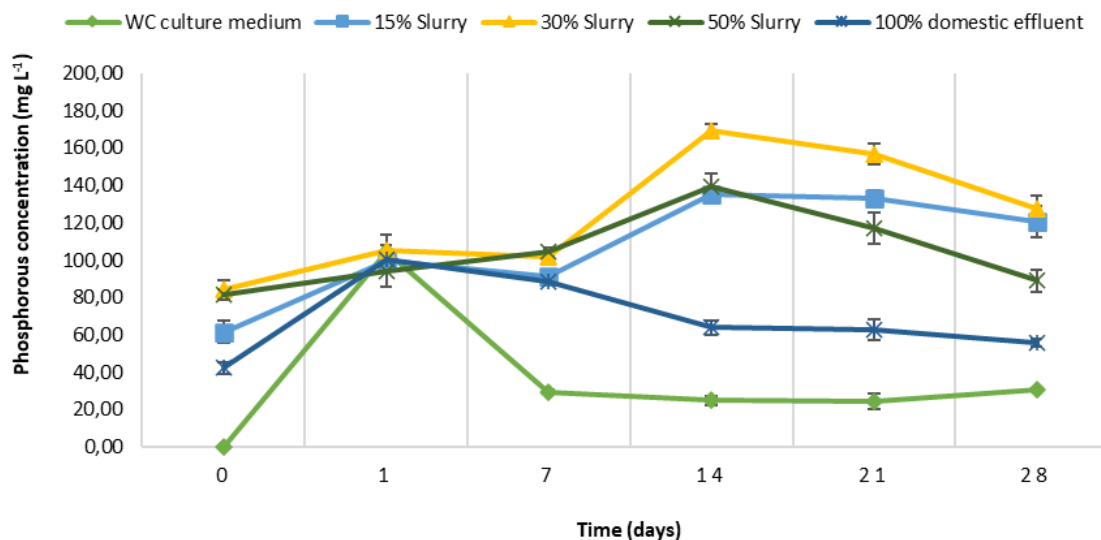
3.4 Phosphorus Removal

Figure 4 shows the results of phosphorus removal over the 28 days of cultivation, in terms of removal pattern, there was no constant reduction during the initial 14 days of cultivation. There was a decrease in the concentration of phosphorus

followed by its increase, generating peaks, and from the 14th day, the removal pattern acquired a constant behavior of reducing the concentration of the phosphorus in the medium. Phosphorus values increase from 0d to 1d, this is because the microalgae strains were grown in nutrient-enriched culture medium and when transferred microalgae were also transferred nutrients. Initially the phosphorus concentrations in slurry in 15%, 30% and 50% are $100,00 \pm 2,10 \text{ mg.L}^{-1}$, $105,40 \pm 2,40 \text{ mg.L}^{-1}$, $90,80 \pm 8,40 \text{ mg.L}^{-1}$, respectively. Considering in 100% domestic effluent containing $100,22 \pm 0,27 \text{ mg.L}^{-1}$ initial phosphorus.

Phosphorus is considered a macronutrient acting as energy (ATP) in the metabolism of microalgae, being important even in small amounts in the culture medium and that may limit the growth of some microalgae species (Lourenço, 2006). The deficiency of phosphorus in the medium produces an increase in lipid content in the composition of dry biomass (Duarte, et al., 2021). Concentrations of 15%, 30% and 50% slurry showed an increase in phosphorus values from the 1st day until the 14th day of cultivation. This fact will be explained in the discussion of efficiency in phosphorus removal. Phosphorus was removed in 100% domestic effluent from $100,22 \pm 0,27 \text{ mg.L}^{-1}$ to $55,80 \pm 2,13 \text{ mg.L}^{-1}$.

Figure 4 - Phosphorus concentration over the 28 days of microalgae cultivation according to the different concentrations of slurry and domestic effluent.



Source: Authors (2022).

3.5 Discussion of phosphorus removal and efficiency

Phosphates are classified as: orthophosphates, hydrolyzable phosphates (pyro-, meta- and other polyphosphates) and organically bonded phosphate. The order of phosphorus forms used by microalgae is orthophosphate > pyrophosphate > metaphosphate > creatine phosphate (Pereira & Branco, 2007; Martinez, et al., 2000), it is believed that there was the excretion of phosphorus in other sources being released in the medium through cell rupture, this fact is observed with the increase of phosphorus concentration in 15% slurry, leaving from $100,00 \pm 2,10 \text{ mg.L}^{-1}$ to $124,47 \pm 8,58 \text{ mg.L}^{-1}$, and 30% slurry, from $105,40 \pm 2,40 \text{ mg.L}^{-1}$ to $127,40 \pm 7,47 \text{ mg.L}^{-1}$, not presenting phosphorus removal efficiency.

However, phosphorus was removed in other concentrations and up to 80.51% the crop containing the WC medium (control) which is where the optimal condition for microalgae growth is found. Other photobioreactors showed an efficiency range of 6.61% in the 50% slurry concentration and 44.32% in the concentration of domestic effluent (100%) show by Table 5, being below the result achieved in the reference (Marques, et al., 2020) urban wastewater which was up to 70% phosphorus removal for the same concentration of wastewater from urban rivers, however, the initial phosphorus concentrations in the

author's work are lower than the initial concentrations of the present study, being 21.22 mg.L⁻¹ and 6.32 mg L⁻¹, initial and final, respectively, in this study 100.22±0,27 mg.L⁻¹ was identified, reducing to 55.80±2,13 mg L⁻¹, initial and final, respectively, with an efficiency of 44.32%.

Other studies such as Hu et al. (2021), used a pre-treatment of the leachate and then dilution for the use of microalgae in phosphorus removal, which highlighted removal equivalent to 80%, however, the greater the slurry dilution, the lower the phosphorus removal rate, corroborating what was observed. was found in this present research.

Table 5 - Efficiency in phosphorus removal (mg L⁻¹) in different slurry concentrations and in 100% domestic effluent. Data with mean and standard deviation (n=3).

Concentrations	Initial phosphorus (mg.L ⁻¹)	Final phosphorus (mg.L ⁻¹)	Efficiency (%)
WC culture medium (Control)	104,67±8,84	20,40±0,05	80,51%
15% Slurry	100,00±2,10	124,47±8,58	0%
30% Slurry	105,40±2,40	127,40±7,47	0%
50% Slurry	90,80±8,40	84,80±5,64	6,61%
100% Domestic Effluent	100,22±0,27	55,80±2,13	44,32%

Source: Authors (2022).

3.6 Bioeconomy and urban and regional development

The economic and environmental scenario especially in the last two decades has caused much of the metropolises around the world to engage in initiatives to improve infrastructure urban services, based on increasingly sustainable economic models (Oliveira, et al., 2019; Jong, et al., 2015). From of urban and regional development, biotechnologies applied the recycling of nutrients such as nitrogen and phosphorus, bioremediation of effluents with different mechanisms (Duarte, et al., 2021) and the biofixation of greenhouse gases (GHGs) represents a paradigm shift, transition to economic model based on the exploitation of waste inputs with positive impacts not only on the environment but also on public health and economy (Tsui, & Wong, 2019; Kothari, et al., 2019; Lopes, 2015).

Aiming at this great concern in removing contaminants concentrated in urban effluents, some systems are improved to optimize the treatment of landfill leachate with the lowest possible costs, as was a system of this research (Md, et al., 2019), where it proved the 80% reduction in biochemical demand of oxygen as to the chemical use of oxygen, among other parameters.

The idea of using a biological treatment system and mainly based on microalgae, consists of acquiring this reduction in the removal of toxic contaminants, but also in the generation of biomass with added value and potential for the generation of bioproducts. These bioproducts can even be sold helping to pay the costs in the entire treatment system. Microalgae can be used as biofuels (biomethane, biodiesel and bioethanol) (Xiao, et al., 2019; Maia, et al., 2020) and even as biopolymers for the development of bioplastics, which can move the regional socioeconomic scenario (Marques, et al., 2020; Duarte, et al., 2021).

4. Conclusion

The microalgae *Chlorella vulgaris*, was able to adapt and grow in different concentrations of cultivation in the leached medium of Landfill diluted with domestic effluent. The increase of a source of phosphate to cultivation contributed to the microalgae growth in concentrations of up to 50% of slurry. The best results of algal growth were obtained in

photobioreactors containing 100% domestic effluent and 15% of slurry with chlorophyll concentration respectively of $2277,08 \pm 247,21 \mu\text{g.L}^{-1}$ and $223,12 \pm 17,44 \mu\text{g.L}^{-1}$ on the 21 day of cultivation.

From the results obtained, it was possible to observe that the microalgae *Chlorella vulgaris* has a great biotechnological potential in the treatment of liquid residues, about the removal of nutrients of interest nitrogen and phosphorus, the specie used in this study presented a good efficiency in this condition, about 72,36% for nitrogen in 50% of slurry and 44.32% for phosphorus in 100% of domestic effluent at the end of the growing time. It is suggested that further research be carried out testing leachate pre-treatment for greater growth of microalgal biomass and that this biomass be subjected to bioproduct generation tests, mainly biofuels and bioplastics.

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